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Economic comparison of food protein production with single-cell organisms from lignocellulose side-streams

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ABSTRACT

In recent years, the cellular agriculture concept has been proposed as an option to replace livestock proteins. This study presents a conceptual level techno-economic analysis of four concepts where food is produced by microorganisms based on wheat straw. Three single-cell proteins and one recombinant protein process were conceptualized. The process included steam explosion pretreatment, enzymatic hydrolysis, fermentation, and downstream processing. The enzymatic hydrolysis was optimized to minimize the production costs. The minimum protein selling price was determined by net present value using the discounted cash-flow method, which was compared to respective animal and plant proteins. The minimum protein selling prices were 5160–9007 €/ton proposing the processes to be in the feasible range, but the processes still require further development. The sensitivity of the parameters was estimated by sensitivity analysis, which revealed the most critical components in the production to be capacity, investment, interest, and enzyme and raw material costs.

1. Introduction

Protein consumption is increasing due to population growth and lifestyle changes (Boland et al., 2013). The demand for meat and dairy protein is expected to grow by 40% and reach 1500 million tons by 2050 (McLeod, 2011). Livestock production has a massive environmental footprint, requiring an increase of 74-fold land and 8-fold freshwater resources, while producing 25 times more GHG emissions compared to plant-based proteins (Poore and Nemecek, 2018). One emerging technology that has the potential to decrease the environmental impact of protein production is fermentation-based "cellular agriculture", in which microorganisms are used to produce food ingredients (Rischer et al., 2020).

Cellular agriculture enables the production of substitutes for traditional protein sources for human consumption. Microbial proteins can be classified as single-cell proteins (SCPs) when the whole cellular biomass is utilized as a food ingredient (Ritala et al., 2017) or as acellular proteins when the proteins are produced by the microorganism and are separated from the cellular biomass (Dance, 2017). SCPs have a high protein content, typically 30–65% depending on the organism, and suitable amino acid composition for food use (Bajpai, 2017; Ekmay, 2019; Ritala et al., 2017). In the 1960s, a *Fusarium venenatum* was observed to have a texture similar to meat and was successfully brought to market in 1985 with a trademark Quorn™ and is nowadays the most known SCP product (Ritala et al., 2017; Wiebe, 2002). Another example of commercially successful SCP is the "Pekilo" process, which operated in the '70–'80s in Finland, using the filamentous fungus *Paecilomyces variotii* for producing feed protein from the spent liquors of sulfite pulping plants (Forss et al., 1986). The third type of SCP called Torula yeast (*Candida utilis*), also shows enormous potential as it already has food regulatory approval (Ekmay, 2019).

The second category of protein products in cellular agriculture is acellular proteins, which are often produced in genetically engineered microorganisms and secreted to the fermentation medium (Stephens et al., 2018). These products include drop-in substitutes for animal-based proteins, such as milk and egg proteins. Recently, several start-up companies have been starting to produce acellular food proteins (Dance, 2017). The cellular agriculture concept and the use of side-stream based raw materials in the food production has raised increasing interest in recent years (Ekmay, 2019; Souza Filho et al., 2018; Upcraft et al., 2020).

Earlier studies have introduced the potential of utilizing side-stream -based feedstocks, such as lignocellulosic feedstocks, in microbial feed and food protein production (Bajpai, 2017; Ekmay, 2019). Lignocellulose is the most abundant raw material on Earth and, for example only wheat straw is globally produced 865 million tons *per annum* (Alakangas

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et al., 2016; Bajpai, 2017; FAOSTAT, 2018). The use of lignocellulosic feedstocks for food-grade microbial protein production is aspiring as it is decoupled from the food production in contrast to the traditional processes where the feedstock is glucose (Angenent and Molitor, 2019). Besides, food protein production from lignocellulosic side-streams provides a possibility to produce a higher-value product compared to a 2nd generation biofuels and chemical production (Bajpai, 2017; Pihlajaniemi et al., 2020). Utilization of lignocellulosic biomass as sugars requires pretreatment and enzymatic hydrolysis of the material (Bajpai, 2017). The pretreatment increases the material's hydrolyzability (Humbird et al., 2011), and enzymatic hydrolysis converts oligomeric carbohydrates to monomeric sugars (Bajpai, 2017). Steam explosion, a commonly used pretreatment method at an industrial scale (Humbird et al., 2011) dissolves hemicelluloses and modifies lignin (Singh et al., 2015) making the feedstock more accessible for the enzymatic hydrolysis.

Since enzymes are a key cost of the process (Ritala et al., 2017), it is important to optimize hydrolysis conditions such as enzyme dosage, hydrolysis time, and solids concentration to minimize the production cost of lignocellulosic sugars. Previous reports of techno-economic analysis (TEA) of lignocellulose hydrolysis have relied on predetermined scenarios of hydrolysis conditions and yield, without cost-optimization (Humbird et al., 2011; Johnson, 2016; Klein-Marcuschamer et al., 2012; Liu et al., 2016). Furthermore, sensitivity analysis of each variable has been carried out separately, without linking yield to the conditions. In previous work by the authors, an empirical hydrolysis model was provided for linking enzyme costs to the corresponding yield (Pihlajaniemi et al., 2020). However, since hydrolysis time and solids concentration mainly contribute to investment costs, they can only be optimized as a part of a TEA of a complete sugar production line investment. So far, the integration of hydrolysis cost-optimization to TEA has not been reported.

This article aims to determine the economic potential of microbial protein production from wheat straw utilized as a food protein. A conceptual level techno-economic analysis is carried out to assess the minimum protein selling price (MPSP) of well-known SCPs, Pekilo (*Paecilomyces variotii*), Torula (*Candida utilis*), and Fusarium (*Fusarium venenatum*), and a generalized acellular recombinant protein using lignocellulosic sugars as the carbon source. In earlier studies, similar analyses have been made for feed SCPs (Pihlajaniemi et al., 2020), whereas this is the first report evaluating the technical and economic potential of food SCPs and recombinant protein from lignocellulose feedstocks. Furthermore, this study incorporates automated cost-optimization of hydrolysis conditions into the TEA for the first time according to our knowledge.

2. Materials and methods

2.1. Feedstock and production processes

Wheat straw was assumed to have a dry matter (DM) of 85% and to contain 37% cellulose, 27% hemicelluloses, 20% lignin, and other 16% components (Alakangas et al., 2016). The base case wheat straw price was estimated as 42.57 €/DM ton using an internal information and by calculating the transportation costs.

The process from straw to the product consists of three main steps: 1) lignocellulosic sugar production, 2) fermentation, and 3) downstream processing (Fig. 1). The first step, lignocellulosic sugar production, is similar to all processes while the fermentation and the downstream processing (DSP) differ by the process. The fermentation is unique for SCP and recombinant protein process and the DSP differs by the product.

2.1.1. Lignocellulosic sugar production

Lignocellulosic sugar production from wheat straw consists of steam explosion pretreatment, enzymatic hydrolysis of the pretreated slurry, and solid-liquid separation of the sugars. The final product from lignocellulosic sugar production, sugar-rich hydrolysate, is used as a carbon source in the upcoming fermentation processes. Lignocellulosic sugar production was designed according to literature references (Humbird et al., 2011; Niemi et al., 2017; Pihlajaniemi et al., 2020).

The process initiates as the straw is shredded, mixed with a 1% food-grade H_2SO_4 solution (Niemi et al., 2017) to 30% DM, and heated in a preheater to 100 °C (Humbird et al., 2011). Next, the slurry is led to a continuous steam explosion reactor, with a retention time of 15 min at a temperature of 190 °C (Niemi et al., 2017; Pihlajaniemi et al., 2020) and a 5.57 bar pressure by adding 13 bar high-pressure steam (Humbird et al., 2011; Pihlajaniemi et al., 2020). The high-pressure steam consumption in the process was estimated as 0.4 ton per ton DM, as suggested by the authors of Humbird et al. (2011). The pretreated slurry is discharged to a flash tank, where the slurry is cooled by flashing the steam, and then the cooled slurry is transferred to a neutralization tank, where food-grade NaOH (50%) neutralizes the excess H_2SO_4. Finally, the pretreated slurry is transferred to enzymatic hydrolysis, where the reaction temperature is 45 °C (Niemi et al., 2017). After hydrolysis, solids are separated from the hydrolysate by centrifugation and washed once to recover sugars from the solid-bound liquid. Pentose and hexose

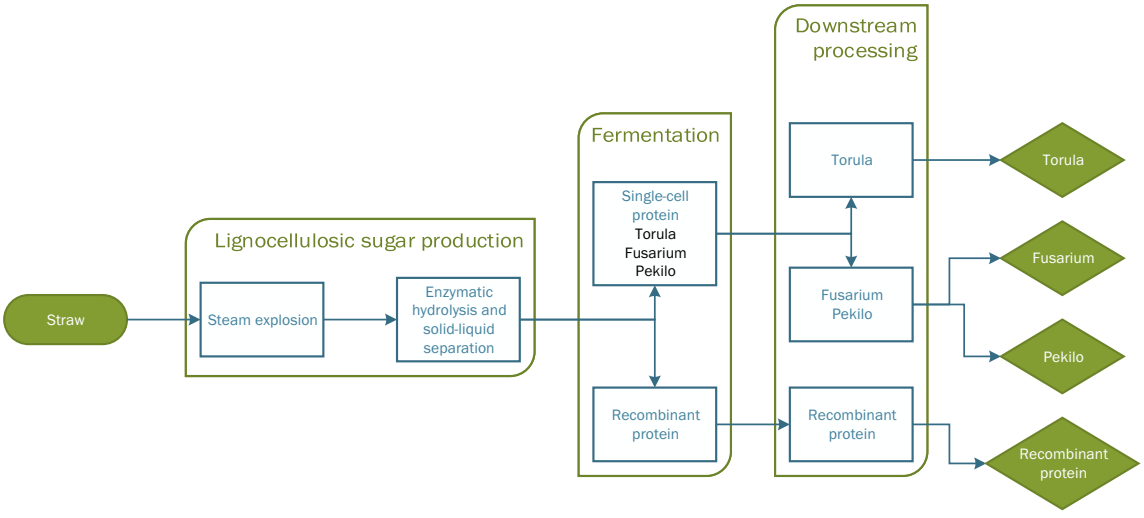

Fig. 1. The value chain from straw to four microbial protein products (Pekilo, Fusarium, Torula, and generalized recombinant protein) consist of lignocellulosic sugar production, fermentation to proteins, and downstream processing.

conversions from cellulose and hemicelluloses during the steam explosion were 0.44 and 0.05, respectively (Pihlajaniemi et al., 2020). NaOH was used to neutralize the excess H_2SO_4 and the consumption was calculated by the molar fractions.

2.1.2. Single-cell protein production

Three SCP processes Torula (*Candida utilis*), Pekilo (*Paecilomyces variotii*), and Fusarium (*Fusarium venenatum*) were designed based on similar processes (Angenent and Molitor, 2019; Harris, 1949; Pihlajaniemi et al., 2020) and assumptions. All SCP processes are working under continuous operation and the fermentations follows the same design. Hydrolysate from the enzymatic hydrolysis is applied for medium preparation, where nutrients and water are added (Humbird et al., 2011), and the medium is sterilized using a steam injector. The sugar concentration was set at 50 g/L (Angenent and Molitor, 2019) in all SCP fermentations. The sugar concentration in the hydrolysate was 67.7 g/L for the base case scenario, thus requiring dilution before SCP.

Pentose and hexose sugars were assumed to be utilized by the microorganisms, assuming 90% utilization of the whole sugar content in the broth, with biomass conversions of 0.5 (Forss et al., 1986), 0.31 (Angenent and Molitor, 2019), 0.37 (Harris, 1949) for Pekilo, Fusarium, and Torula, respectively. The conversion to Fusarium included 30% biomass loss due to the RNA reduction that is needed to obtain a food-grade product (Angenent and Molitor, 2019) and the same 30% biomass loss was applied for Pekilo and Torula for ensuring food safety. During the fermentation, food-grade NH_4OH (25%) is used to provide nitrogen and pH-control. The microorganism's nitrogen requirement was calculated by dividing the protein content in biomass by 6.25 and corresponding NH_4OH consumption by molar fractions (Pihlajaniemi et al., 2020).

The SCP Torula differs from the Pekilo and Fusarium as not being filamentous. The DSP of the Torula was designed from the old Torula process (Harris, 1949; Bajpai, 2017); thus, the separation of the SCP is done by using centrifugation, whereas Pekilo and Fusarium are separated using pressure filtration (Bajpai, 2017; Forss et al., 1986). The final DM after pressure filtration and centrifugation were set to 0.25 (Aden, 2003; Bekatorou et al., 2006). All SCPs are dried to 90% DM by a fluidized bed dryer.

2.1.3. Recombinant protein production

One generalized recombinant protein process based on the production of microbial egg protein was designed mainly using VTT's technical data and following the U.S's Natural Renewable Energy Laboratory's (NREL's) cellulase fermentation process and assumptions, that describes the production of cellulase by *Trichoderma reesei* in a fed-batch process (Humbird et al., 2011). The NREL's process set up is applicable to recombinant food protein production as the production host is the same and the product similar. Hydrolysate from the enzymatic hydrolysis is used for the medium preparation and nutrients are added (Humbird et al., 2011), and the medium is sterilized with a direct steam injector. The sugar concentration in the hydrolysate was 67.7 g/L for the base case scenario, and thus required concentration to 200 g/L.

The recombinant protein fermentation is a fed-batch process and contains a seed train with three seed fermenters 0.3, 3, and 30 m^3, which provides seed for 300 m^3 main fermenters. Antifoam solution is fed continuously during the recombinant protein process to decrease undesirable foaming. The biomass and protein conversions from sugars were assumed to be equal, 0.2 for the recombinant protein process and the initial sugar concentration in the recombinant protein fed-batch fermentation was 200 g/L (Ellilä, 2020). The NH_4OH supplies nitrogen and pH-control. The microorganism's nitrogen requirement was calculated by dividing the protein content in biomass and extracellular protein by 6.25 and corresponding NH_4OH consumption by molar fractions (Pihlajaniemi et al., 2020).

The recombinant protein DSP includes two filtrations; the first is a pressure filtration that removes the cells, and the second is a sheet filtration that is used further to purify the protein in aims to remove all remaining cells and impurities (Doran, 2013, pp. 447–450). The yield from filtrations were 0.93–0.95 (Ellilä, 2020; Kujanpää, 2020). Next, the protein is concentrated to 28% (Kujanpää, 2020) using ultrafiltration and finally dried to 90% DM using spray drying (Humbird et al., 2011; Kargi and Shuler, 2014).

2.2. Design basis and assumptions

The process was designed based on an annual raw material capacity of 40,000 DM tons of straw with 8000 h of an annual production time. The process balances were calculated in Microsoft Excel. A material loss of 2% was assumed for each process step. The utility requirements, including steam, electricity, chemicals, and process and cooling water, were based on material and energy balance calculations. The heating was provided as 5.5 bar low-pressure steam (Pihlajaniemi et al., 2020) and cooling as by cooling water. Electricity consumption was calculated from the agitation and aeration demands, and an additional 20% was included to cover the other electricity needs. A major part, 80%, of the process water was recycled from wastewater streams and purified in tertiary wastewater treatment. All reactors were designed assuming a 70% degree of filling, and the chemical storages were designed to store chemicals for 30 days use.

2.3. Hydrolysis model and automatization of hydrolysis optimization

An empirical hydrolysis model, describing an asymptotic response to enzyme dosage and hydrolysis time, and a linear negative response to solids concentration (Eq. (1) (Pihlajaniemi et al., 2020)) was integrated into TEA to give a continuous yield response to changing hydrolysis parameters. The model parameters were originally determined for hydrothermally pretreated grass silage fiber, which was considered comparable to wheat straw in composition and hydrolyzability. Enzyme dosage, hydrolysis time and solids concentration were optimized by minimizing sugar production cost by linear optimization (*Solver* function) with Microsoft Excel. Optimization was automatized by using VBA-programming to trigger the *Solver*-function upon recalculation of the sheet.

$$Y = (Y_{max} - \beta_1 c)\left(\frac{\beta_2 E}{\beta_2 E + 1}\right)\left(\frac{\beta_3 t}{\beta_3 t + 1}\right)\left(\frac{\beta_4 Et}{\beta_4 Et + 1}\right) \quad (1)$$

2.4. Cost estimation

The total cost of production included operating (OPEX) and capital expenditures (CAPEX). All costs are presented per DM ton of protein content of the SCPs or purified recombinant protein. Operating costs consist of variable and fixed costs. Variable costs that include all costs generated by material and utility consumption, were estimated based on plant material, water, and energy input. Fixed costs include labor and maintenance costs. Labor need was estimated as two workers per process section in continuous processes and three workers in fed-batch operation, working in three shifts (Seider et al., 2009), and the labor fees were assumed as total-cost salaries of 70,000 €/a including all employee fees (Pihlajaniemi et al., 2020). Maintenance costs were assumed as 1.5% of the total capital investment (Pihlajaniemi et al., 2020).

Capital investment was estimated using the factorial method (Peters and Timmerhaus, 1991) in which the purchase cost of the equipment was scaled using scaling factors from the literature (Humbird et al., 2011; Mujumdar et al., 2015; Towler and Sinnott, 2012). The purchased equipment was identified as reactors, tanks, centrifuges, filtration systems, dryers, agitators, compressors, and process, cooling, and wastewater systems. An additional 20% was added to the estimated purchased equipment costs for other equipment, such as pumps and conveyors. For SCP process an additional 10% cost was assumed as an extra reactor is needed for RNA removal. The equipment quote prices from earlier

reports were converted to 2019 prices using the chemical engineering plant cost index (CEPCI) that was on average 607.5 (ChemEng online, 2020). Any distinct location was not specified for the plant and the costs were obtained from Finnish and global sources. Working capital was set to 5% of fixed capital investment (Humbird et al., 2011; Shafiei et al., 2013).

2.5. Economic analysis

The economic potential of the protein production processes was estimated by calculating minimum protein selling prices for the products. The price was determined by calculating zero net present value based on the discounted cash flows. The revenue consisted of the protein sales and the process waste utilization, including technical loss, solid residue from hydrolysis, and cell waste in the recombinant protein process. Waste utilization value was evaluated based on the material's net energy content by combustion.

Discounted cash flows to the firm were calculated using 20-year amortization. The plant was financed with 40% equity and 60% debt assuming an average 5% interest rate. The investment was split into two installments during a two-year construction period. First-year, 30% of the FCI was invested, and the rest 70% during the second year. A one-year start-up period was assumed, during which a 50% revenue was achieved, requiring 50% variable costs and 100% fixed costs. The MPSP's were solved iteratively using the *Goal Seek* function of Microsoft Excel, which was automatized by VBA programming. The MPSPs were compared to respective commercial plant product sales and animal protein prices.

Parameter uncertainty was assessed using Monte Carlo analysis built into the VBA code, so that each scenario would also include the iterative hydrolysis optimization and MPSP iteration. The Monte Carlo analysis is an overarching assessment in which random numbers are generated to present the uncertainty of the selected parameters within the determined range by running multiple simulations and calculating the overall probability distributions, thus allowing to estimate several process parameters easily (Humbird et al., 2011; Towler and Sinnott, 2012, pp. 416–417).

Uniform distribution was used for all parameters, except enzyme price (Table 1). For enzyme price of 10,000 €/ton base case price was assumed (Niemi et al., 2017; Pihlajaniemi et al., 2020) and a triangle distribution was used since there was a better *a priori* knowledge based on industry expertise. For other parameters, a rather wide range was used, so that it is most likely that the actual cost is covered within the uncertainty assessment.

3. Results and discussion

3.1. Optimization of enzymatic hydrolysis

Incorporation of a hydrolysis response model (Eq. (1)) to TEA allowed optimization of the enzyme dosage (E), hydrolysis time (t) and solids concentration (c) by minimizing production costs of sugars. From a range of inflation-corrected enzyme price estimates of 6–20 €/kg protein for commercial enzymes (Johnson, 2016; Liu et al., 2016), and 3–10 €/kg (Ellilä et al., 2018; Johnson, 2016; Klein-Marcuschamer et al., 2012) for an on-site enzyme production, 10 €/kg protein was selected as the base case. The optimum hydrolysis yield for the base case was 57.53%, with the optimum E of 5.07 mg/g DM (9.07 mg/g cellulose), optimum t of 128.52 h and optimum c of 12.80%. The optimum hydrolysis yield and dosage were considerably lower compared to the frequent assumption of 90% yield with a dosage of 20 mg/g glucose (Humbird et al., 2011; Klein-Marcuschamer et al., 2012), suggesting that abandoning a fixed yield target can in fact improve the feasibility of the process, even if the optimum is below expectations.

In order to study how the optimum hydrolysis conditions are affected by changes in enzyme price and equipment costs, hydrolysis optimization was automatized to maintain optimal hydrolysis conditions throughout all changes in other variables. Hydrolysis CAPEX was defined as the investment costs affected by hydrolysis time and solids concentration. The benefit of integrating hydrolysis optimization into TEA can be illustrated by comparing the production costs of automatically optimized hydrolysis with base case hydrolysis and their sensitivity to changing enzyme and equipment prices. The farther the enzyme price (Fig. 2A) and hydrolysis CAPEX (Fig. 2B) shift from the base case, the larger the reduction of production cost by optimization becomes. The sugar production cost were in the range of 460–550 €/DM ton by changing the enzyme price and hydrolysis CAPEX 50–150% from the base case. The optimum hydrolysis yield correlates negatively with hydrolysis costs, since higher enzyme dosages and longer hydrolysis times are affordable at lower enzyme prices and equipment costs. At enzyme prices of 25 and 200% of the base case, the optimum yield ranges from 67 to 51% and the optimum E decreases from 11.2 to 3.3 mg/g DM. The decreased E is partially compensated by an increase in optimum t. In turn, increasing hydrolysis CAPEX decreased optimum t and increased optimum E. However, the optimum c was increased by raising both enzyme as well as hydrolysis CAPEX.

Since the cost of t and c are both mainly realized through the investment costs of the equipment whose scale is affected, their interdependency was mapped in more detail. Fig. 2C presents the sugar production cost as a function of t and c, with automatically optimized E. Sugar production costs below 520 €/ton could be reached with a solid's concentration of 7.5–20% DM or a hydrolysis time of 60–192 h. The cost was less sensitive to t, which only affects the hydrolysis reactor's size, whereas c also affects the amount of water and hydrolysis slurry to be processed, and consequently the scale of subsequent processing equipment. The sugar production costs were considerably higher compared to previous estimates of 308–430 €/ton with similar processes, where higher yields and lower enzyme prices were assumed (Tao et al., 2013), and compared to the 10-year average sugar market price of 308 €/ton (IndexMundi, 2020). Solids concentrations of 20% are often considered industrially relevant, particularly in cellulosic ethanol concepts (Humbird et al., 2011; Klein-Marcuschamer et al., 2012). However, as opposed to cellulosic ethanol production, protein production does not allow simultaneous saccharification and fermentation, which constrains the applicable c range.

Table 1
The parameters, selected distributions and ranges used in the Monte Carlo analysis of the four protein production processes.

Parameter	Distribution	Min	Base case	Max
Enzyme price, €/ton	Triangle	3000	10,000	20,000
Interest, %	Uniform	1	5	7
Investment, %	Uniform	75	100	125
Capacity, DM ton/a	Uniform	20,000	40,000	60,000
Raw material cost, %	Uniform	50	100	150
By-product valorization, %	Uniform	50	100	150
Energy and electricity, %	Uniform	50	100	150

3.2. Financial comparison of proteins

After applying the optimal hydrolysis conditions, the minimum protein selling prices for Pekilo, Fusarium, Torula and recombinant protein were 5160 (4317–5949), 6549 (5561–7439), 7311 (6246–8354), and 9007 (7956–10,049) €/ton, respectively (Fig. 3A). The MPSP's are in the range of commercial food protein products if compared to plant-based protein products (av. 7400 €/ton) and egg and milk protein ingredients (av. 10,500 €/ton) (Fig. 3C). The MPSPs represent the wholesale protein prices, thus excluding the final formulation of the products and considering the activities to ensure food safety. However, the calculated MPSPs for the microbial protein products were estimated as mere protein, not as part of the formulated product as in the commercial products. The mere protein costs for the

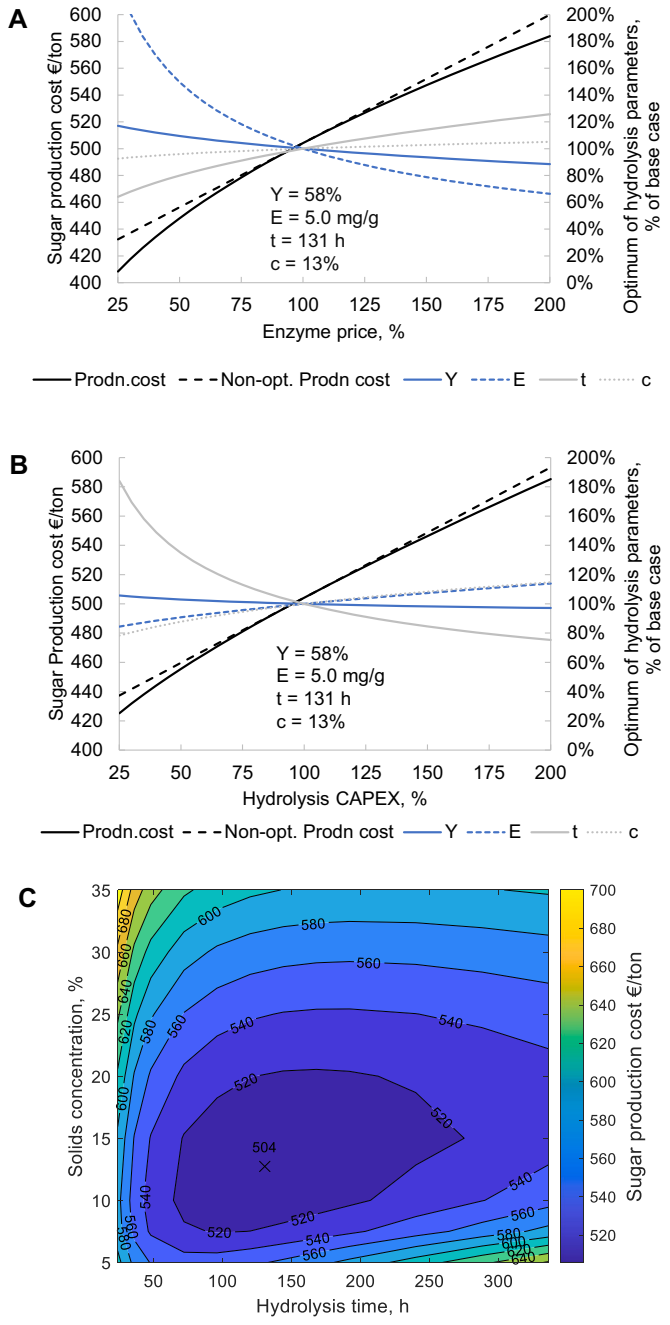

Fig. 2. The optimum of hydrolysis yield, enzyme dosage E, hydrolysis time t and solids concentration c as a function of A) enzyme price and B) CAPEX of hydrolysis reactors and subsequent separation equipment. The corresponding sugar production cost is shown with and without hydrolysis optimization. C) Dependence of sugar production cost on hydrolysis time and solids concentration, when only enzyme dosage is optimized.

commercially formulated products are up to 2–10 times higher than the calculated MPSPs (Fig. 3C). Price comparison between current commercial products and MPSPs implicates considerable sales margin even at current technology and strains. Comparing the actual cost of protein, the production seems economically viable even though the product formulation will increase the total costs. Formulation costs were outside the scope of this article.

Comparing the minimum selling prices of similar processes is difficult since there are not many studies available according to our knowledge. Upcraft et al. (2020) studied the Quorn™ process economics and estimated a minimum selling price for Fusarium paste of 6250–7470

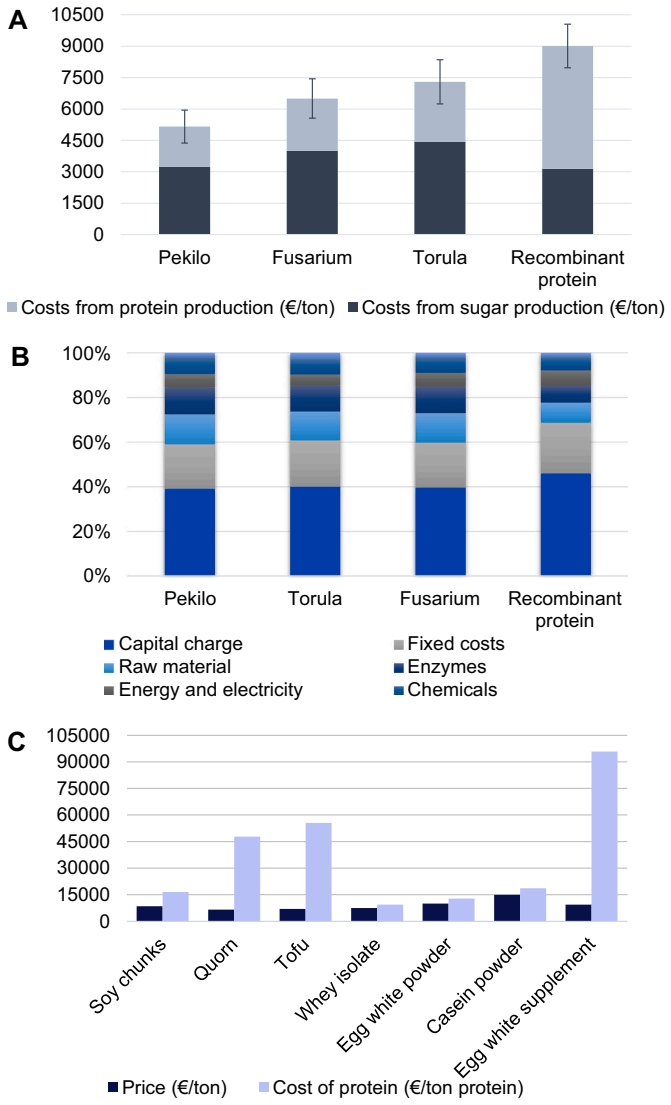

Fig. 3. Minimum selling prices (A), main cost components in the manufacturing (B) of Pekilo, Torula, Fusarium, and generalized recombinant protein from lignocellulosic sugars produced from wheat straw and consumer selling prices and protein costs for commercial protein products (C) (Alibaba, 2020a, 2020b; Fitnesstukku, 2020; Foodie, 2020a, 2020b, 2020c; Tesco, 2020).

$/ton (54350–64,960 $/ton protein), which was considerably higher compared to the values in this article. However, they mimicked the Quorn™ process with over 10-fold purchase costs for fermenters and separation equipment and estimated higher capital costs for RNA reduction equipment. The biomass reduction caused by the RNA reduction was included but with lower capital costs. The authors of Pihlajaniemi et al. (2020) studied the production of feed Pekilo SCP from grass silage fiber and suggested the protein price to set slightly over 2000 €/ton, when SCP was combined with protein extracted from the feedstock, thus changing the economics compared to our study. Also, the RNA reduction increases the costs and the development of food products requires a suitable texture and thus the final production might require texturizing operations comparable to the Quorn™ process. Texturization costs were not estimated in this study, which will add the costs for formulation.

The major cost components in all single-cell and recombinant protein processes are the same, including the capital, fixed, raw material, and enzyme costs (Fig. 3B). As expected, a considerable part of the costs comes from processing the raw material into sugars, including the raw

material and enzyme cost and the related capital costs, which contributes 35.1–62.8% of the total manufacturing costs. The contribution of variable operating costs, including all necessary raw materials and utilities, are 31–41% and the fixed expenses are 20–23% of the total production costs, and the residual 39–46% comes from the capital costs. Capital costs are higher for the recombinant protein process than SCPs since the fed-batch production requires more fermenters and more complex downstream processing is needed for purification of soluble protein and removal of the genetically modified production organism. Also, among the SCPs the capital costs are slightly higher for the Torula process as the non-filamentous structure requires expensive separation by centrifugation compared to filamentous Pekilo and Fusarium (Angenent and Molitor, 2019; Bajpai, 2017; Forss et al., 1986) which partly explains the higher production costs of the Torula compared to Pekilo. Torula also has the lowest conversion rate from sugars to protein of the SCP after considering the RNA reduction, contributing to the higher MPSP. Fusarium has 2% higher sugar to protein conversion compared to Torula and 4% lower than the conversion of Pekilo which explains the production cost differs between Torula and Pekilo.

The equipment costs of lignocellulosic sugar production are 27–48% of the total equipment cost depending on the product, because of the expensive pretreatment reactor, hydrolysis reactor and centrifuges. Similar results have been observed in the 2nd generation biorefineries, where the processing of the raw material is the most expensive part of the process, attributed to high CAPEX and enzyme cost (Gnansounou and Dauriat, 2011; Humbird et al., 2011; Shafiei et al., 2013). The studies on Fusarium (Upcraft et al., 2020) and Pekilo (Pihlajaniemi et al., 2020) processes introduced similar variable cost structure, raising the cost of raw material and enzymes up to half of the total operating costs. A promising alternative to lower the enzyme costs would be by applying the on-site enzyme production to reduce the commercial enzyme costs from 10 to 20 €/kg (Pihlajaniemi et al., 2020) to as low as 3.8 €/kg (Humbird et al., 2011). Further improvements could be gained by evaluating other well established (Balan et al., 2013) or exploratory (Maroušek, 2012) pretreatment technologies for synergy with food grade SCP fermentation, and applying advanced data-based technologies for biomass logistics and process control (Peters et al., 2020). The overall contribution of cost components resembles similar TEAs (Gnansounou and Dauriat, 2011; Humbird et al., 2011; Pihlajaniemi et al., 2020), except for labor costs, which are somewhat higher in the current study.

Even though the production seems feasible at the conceptual level estimate, several research questions are still to be considered. From the economic point of view, the most important one is the cost of the final formulation and approval cost for food-grade safety. Besides, the costs were acquired from several references without assuming any final location and thus the real costs will alter. Yet, the aim in conceptual level study is to estimate the costs roughly to find out the profitability and the more precise costs will be determined if the processes are later built. Also, comparing the product's value to commercially available products is challenging since direct information on the prices of exactly similar proteins is not publicly available and it is difficult to estimate future price development.

3.3. Mass flows

The mass flows of Torula representing the SCP, and recombinant protein process are presented in Fig. 4. The sugar yield from 40,000 DM tons of straw was 15,417 tons/a. Further, the protein mass yield of Pekilo, Torula, Fusarium, and recombinant protein were 2444, 1779, 1972, 2508 DM tons/a, respectively. Utilization of 90% of total sugars was assumed for simplicity, although the ability to use different sugars,

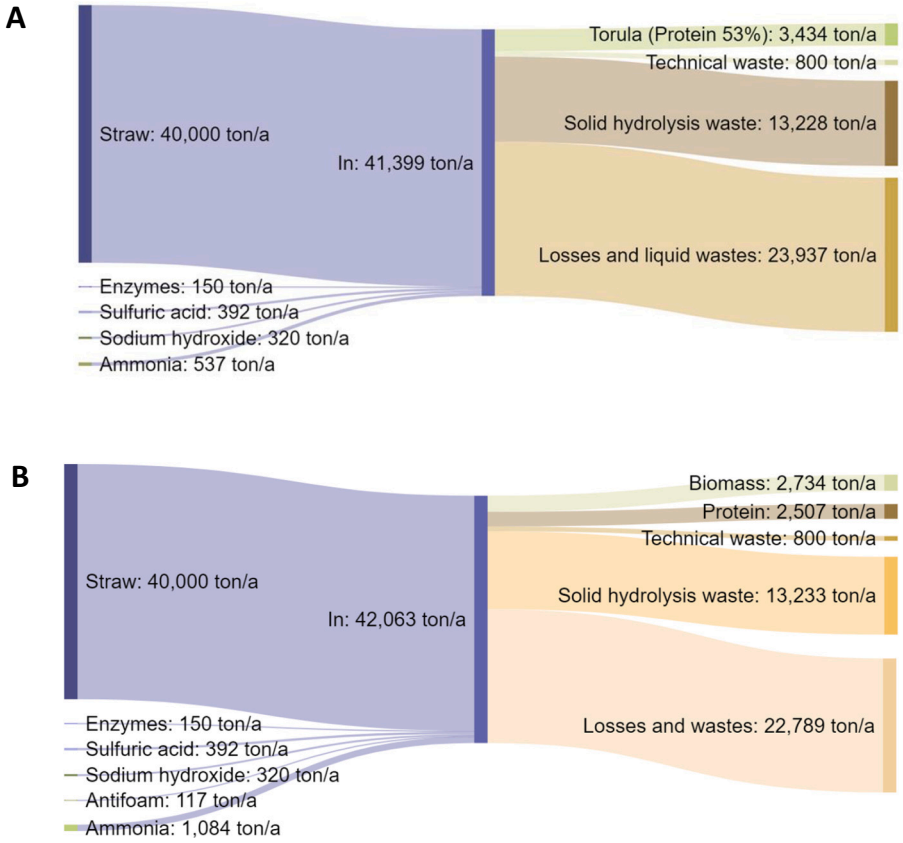

Fig. 4. Sankey diagrams of the annual mass flows of Torula (A) and recombinant protein (B) production. The production process consists of steam explosion pretreatment and enzymatic hydrolysis to lignocellulosic sugars, and fermentation and downstream processing to proteins.

particularly pentoses, will vary among production organisms. The highest yield was for the recombinant protein and Pekilo because the conversion rates were the highest. In turn, the mass yield of Fusarium and Torula were remarkably lower compared to Pekilo and the recombinant protein. Comparing SCPs, the mass yield explains the production costs well as the highest yield generates the lowest MPSPs and contrarily. The same explanation does not apply to the recombinant protein process. The recombinant protein's mass yield was nearly the same as for Pekilo and significantly higher than for Fusarium and Torula, but the MPSP of the recombinant protein was higher than any of the SCPs. The explanation is that the recombinant protein process's capital costs were significantly higher, thus lowering the mass yields relevance. Also, the sugar conversion from straw was estimated based on corresponding values from the processing of grass silage fiber, which has shown hydrolysis yields comparable with other agricultural lignocellulose residues (Pihlajaniemi et al., 2020). However, the exact yields may differ from those of wheat straw.

The process inflows consist of the carbon source, straw, and the rest are chemicals and enzymes used in the lignocellulosic sugar production and NH_4OH in the fermentation. As the carbon source in the process is the most substantial input, the feedstock cost is significant, and ensuring the low price is necessary to keep the production feasible. Overall, the process inflows are akin, the only distinctions are in the NH_4OH feed that is slightly higher in the recombinant protein process than SCPs because of the higher demand for the nitrogen and the addition of antifoam that is necessary for the recombinant protein process.

The outflows of the process consist of the biomass, extracellular protein in the recombinant protein process, and losses and wastes from the production. Sankey diagrams highlight well the important role of the process by-products compared to the main products. The by-products are divided into three categories, solid hydrolysis waste, cell waste in the recombinant protein process, and other process losses and wastes, representing soluble and insoluble, organic and inorganic compounds from the processing. Value increase from the by-products material's net energy content by combustion was 1.5–2.4% of the total revenue depending on the product but could be increased by using more value-added applications such as feed which value can be even up to 1000 €/ton protein (Soy meal, Pihlajaniemi et al., 2020). The *Trichoderma* cell waste from the recombinant protein process can consist of even up to 49.5% crude protein of dry weight (Ahmed et al., 2017). Thus, one ton of the cell waste could have value up to 500 € on a rough estimate. Thus, the value from the cell mass could be increased significantly if considering the feed value rather than the net energy content by combustion.

3.4. Sensitivity analysis

The analysis shows that capacity, investment, raw material cost and enzyme price have the largest impact on the MPSP (Fig. 5). Each process has unique differences in parameter sensitivity. For example, investment and interest have a more significant impact on the recombinant protein and Torula due to the higher capital costs. The raw material annual capacity has the highest effect on the MPSP in all processes due to equipment scaling. Enzyme price has more impact in the SCPs than in the recombinant protein process. In turn, the by-product valorization has the least impact in all processes. The likely total price, described as between low bound as 25th quantile and high bound as 75th quantile of MPSP in 1000 simulations per process were Pekilo 4760–5687, Fusarium 6321–7536, Recombinant protein 8613–9910, Torula 7043–8439 €/ton DM protein. The maximum observed uncertainty was roughly ±9% of MPSP in all processes for selected parameters. Enzyme price's impact is reduced due to the automated enzyme hydrolysis optimization step imbedded in the analysis.

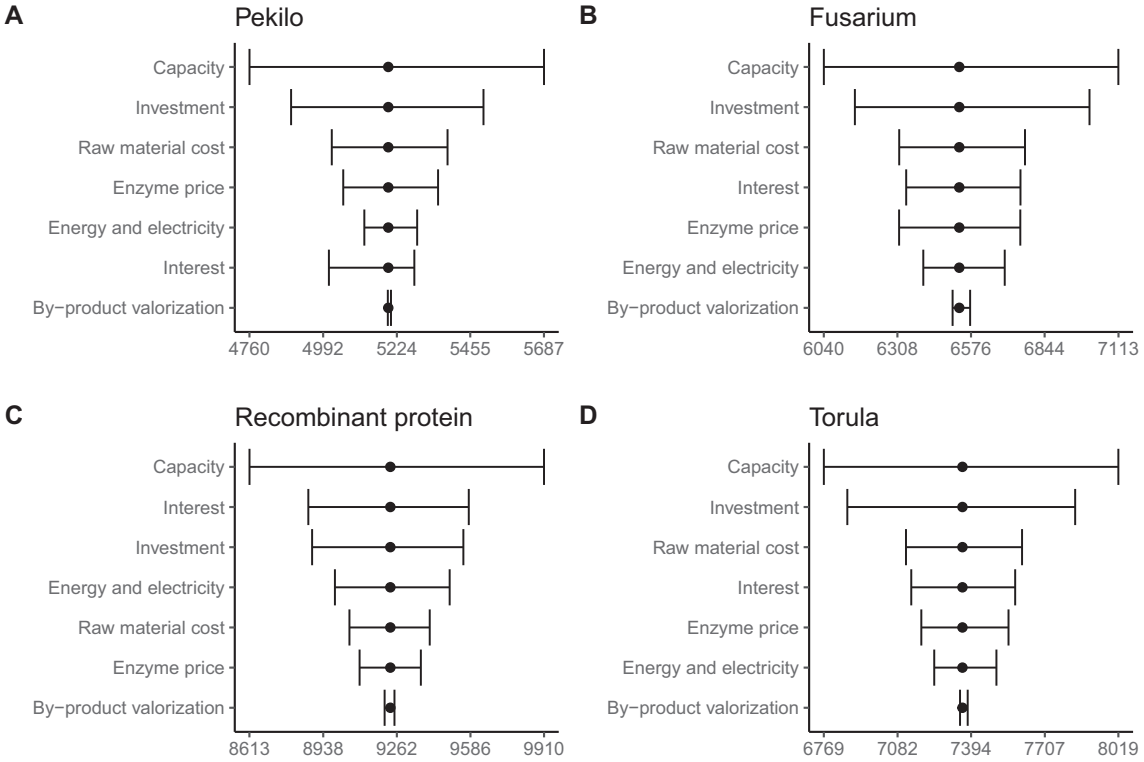

Fig. 5. Quantitative risk analysis for all processes based on Monte Carlo sensitivity analysis: Pekilo, Fusarium, Recombinant protein and Torula, indicated with letters A, B, C and D, respectively. Number of simulations = 1000. X-axis indicates the parameter and Y-axis the minimum protein sales price (MPSP) in €/tons DM. In each of the simulation, hydrolysis is optimized with each set of parameter combination and then the MPSP is calculated. Error bars: The low is indicated by 25th quantile and high with 75th quantile from the simulation results. The point in the middle indicates 50th quantile of the simulations. Uniform distribution ± 50% is used for most parameters, except enzyme price, investment. Details in Supplementary.

3.5. Limitations and food safety

The conceptual level techno-economic evaluation had limitations regarding the production and the products food status. This study focused on evaluating a commercial scale process, whereas the R&D costs for process and strain development and novel food approval costs were not considered.

Fusarium and Torula are accepted within EU (Weatherholtz and Holsing, 1976; Wiebe, 2002) for food use and there are some acellular GMO organisms, such as *T. reesei*, which produces β-lactoglobulin that have GRAS status (FDA 2020, www.fda.gov/media/136754/download) in United States meaning they are allowed in food use. In Europe, Pekilo and recombinant protein would need to pass novel food legislation (EU) 2015/2283 (Rychen et al., 2018). Novel food regulation requires passing a thorough safety testing, including toxicity, risk analysis and compositional analyses, including the description of the process and all original data supporting the approval (Turck et al., 2016). No GMO microorganisms have yet been approved in the EU for food use as protein. However, GMO microorganisms have specific guidance and additional requirements on top of novel food requirements, such as molecular characterization, comparative analysis to non-GMO counterpart, and evaluation of potential environmental impact (Regulation (EU) No 503/2013). The costs of these additional analyses are difficult to estimate and thus were not included.

The cellular agriculture concept and the approach of producing microbial food protein are still fresh and require plenty of R&D efforts to develop the processes and the strains. The calculations were performed in Microsoft Excel as *ex-ante* model that did not provide a platform for analyzing as accurate process data as using simulation tools that take account more specific process parameters, such as device specific heat losses. Thus, these analyses can only be considered as a rough estimation for these novel protein production processes for food, whereas in future research, strain development, process simulation, protein texturizing and approval costs require further attention. The processes included the following actions to obtain the food-grade products; the reduction of RNA from SCPs and adding a sheet filtration to the recombinant protein process that removes all remaining GMO cells. Nevertheless, this study shows considerable potential for food protein production from a lignocellulosic feedstock *via* cellular agriculture, indicating that the high value of food protein offsets the high costs of lignocellulosic sugar production.

4. Conclusions

Conceptual level techno-economic analysis suggested that microbial food protein production from lignocellulosic raw materials could have potential. The yields and minimum protein selling costs of the products varied but were within the range of financial feasibility. The optimization of the enzymatic hydrolysis improved the process economics. Sensitivity analysis identified the main cost drivers such as the plant capacity, investment and raw material costs. However, in the future more attention should be given to strain development, texturization of proteins for food formulation, and food safety authorization.

CRediT authorship contribution statement

Eveliina Voutilainen: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft. **Ville Pihlajaniemi:** Investigation, Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision. **Tuure Parviainen:** Investigation, Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Author Ville Pihlajaniemi is a co-founder, shareholder and from 3rd Jan 2021 employed by the start-up company eniferBio Oy (Finland), which aims to commercialize the production of *Paecilomyces variotii* from industrial (non-lignocellulosic) side-streams and its use as feed (non-food) protein ingredient. All other authors declare no competing interest.

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Appendix A. Supplementary data

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